# PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

## PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

| | |
|---|---|
| Date of mailing: 22 March 2001 (22.03.01) | |
| International application No.: PCT/AU00/01065 | Applicant's or agent's file reference: 102031 |
| International filing date: 08 September 2000 (08.09.00) | Priority date: 10 September 1999 (10.09.99) |
| Applicant: HERBERT, Ben | |

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

15 February 2001 (15.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01065

**A. CLASSIFICATION OF SUBJECT MATTER**

Int. Cl. [7]: G01N 27/447, 27/453, B01D 57/02

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC: G01N 27/447, 27/453, B01D 57/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC AS ABOVE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DWPI, JAPIO

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 9933550 A1 (MACQUARIE RESEARCH) 8 July 1999<br>See figures | |
| A | WO 9857162 A1 (HOEFER PHARMACIA) 17 December 1998<br>See figures | |
| A | WO 9857161 A1 (HOEFER PHARMACIA) 17 December 1998<br>See figures | |

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 2 November 2000 | 29 NOV 2000 |
| Name and mailing address of the ISA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>E-mail address: pct@ipaustralia.gov.au<br>Facsimile No. (02) 6285 3929 | Authorized officer<br>N. STOJADINOVIC<br>Telephone No : (02) 6283 2124 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01065

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | EP 684468 A2 (EASTMAN KODAK) 29 November 1995<br>See figures | |
| A | US 5275710 A1 (GOMBOCZ et al) 4 January 1994<br>See figures | |
| A | US 4443319 A1 (CHAIT et al) 17 April 1984<br>See figures | |
| A | US 5785835 (SAITO et al) 28 July 1998<br>See figures | |

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
**PCT/AU00/01065**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report | | Patent Family Member | | | | | |
|---|---|---|---|---|---|---|---|
| WO | 9933550 | AU | 16530/99 | EP | 1042054 | | |
| WO | 9857162 | AU | 77995/98 | AU | 80629/98 | US | 5989400 |
| | | US | 6113766 | WO | 9857161 | | |
| EP | 684468 | JP | 8043351 | US | 5779869 | | |
| US | 5275710 | EP | 457526 | US | 5104512 | US | 5410412 |
| | | US | 5217591 | | | | |
| US | 4443319 | US | 4483885 | | | | |
| US | 5785835 | NONE | | | | | |

END OF ANNEX

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

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PCT

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(51) International Patent Classification[7]: G01N 27/447, 27/453, B01D 57/02

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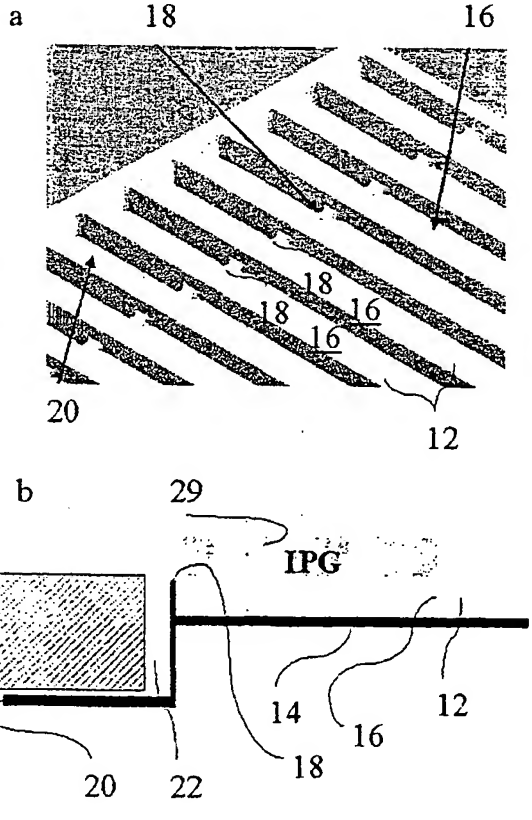
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European

*[Continued on next page]*

(54) Title: ELECTROPHORESIS APPARATUS AND A METHOD OF USING THE SAME




(57) Abstract: An apparatus for rehydrating and for performing electrophoresis on a gel strip includes a tray defining a plurality of parallel troughs configured to receive gel strips. Each trough defines a centrally located rehydration area and an electrode area disposed either side of the electrode area. End walls delimit the rehydration area of the trough from the electrode area. Electrode means including contact points adapted to contact either the gel strip in the electrode areas near the first and second end of the gel or a conducting or current carrying electrode bridge material which is in contact with the gel strip in the electrode areas. The electrode means are adapted to be connected to a means for supplying an electric current for imposing an electric potential in the strip between the electrodes.

WO 01/20315 A1

patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**Published:**
— *With international search report.*

22/3 13

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

REC'D 27 FEB 2001 PCT

(PCT Article 36 and Rule 70)

| | | |
|---|---|---|
| Applicant's or agent's file reference<br>102031 | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416). |
| International Application No.<br>**PCT/AU00/01065** | International Filing Date *(day/month/year)*<br>8 September 2000 | Priority Date *(day/month/year)*<br>10 September 1999 |
| International Patent Classification (IPC) or national classification and IPC<br>**Int. Cl.** [7] G01N 27/447, 27/453, B01D 57/02 | | |
| Applicant<br>PROTEOME SYSTEMS LTD et al | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

| | | |
|---|---|---|
| I | ☒ | Basis of the report |
| II | ☐ | Priority |
| III | ☐ | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| IV | ☐ | Lack of unity of invention |
| V | ☒ | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| VI | ☐ | Certain documents cited |
| VII | ☐ | Certain defects in the international application |
| VIII | ☐ | Certain observations on the international application |

| | |
|---|---|
| Date of submission of the demand<br>5 February 2001 | Date of completion of the report<br>12 February 2001 |
| Name and mailing address of the IPEA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>E-mail address: pct@ipaustralia.gov.au<br>Facsimile No. (02) 6285 3929 | Authorized Officer<br>**N. STOJADINOVIC**<br>Telephone No. (02) 6283 |

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU00/01065

## I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-15 | YES |
| | Claims | | NO |
| Inventive step (IS) | Claims | 1-15 | YES |
| | Claims | | NO |
| Industrial applicability (IA) | Claims | 1-15 | YES |
| | Claims | | NO |

2. Citations and explanations (Rule 70.7)

**Novelty (N) Inventive Step (IS) Claims 1-15**

The documents cited in the international search report are merely indicative on the state of the art and do not throw doubt on the novelty of the invention as claimed.

Specifically, none of the cited documents disclose a rehydration and electrophoresis cell where the electrode does not contact the rehydration solution.

# Electrophoresis apparatus and a method of using the same

This invention relates to an electrophoresis apparatus and to a method of using the same for separating biomolecules by electrophoresis.

## Background of the Invention

Two-dimensional electrophoresis is the preferred method for separating proteins from complex mixtures such as tissue samples, bacteria or plant material. Typically the proteins are separated in the first dimension using an electrophoresis gel with an immobilised pH gradient (IPG). These gels are commercially available and are usually supplied as dry gel strips bonded to a plastic backing sheet. Before the separation takes place the gel must be rehydrated with an appropriate liquid, in which it is ideal to have the protein sample dissolved. The most common embodiment of this approach is to allow the rehydration to occur passively, in a tray comprising a plurality of troughs, until the liquid in each trough has fully rehydrated the IPG gel strip in that trough. This requires that care is taken in selecting the correct volume of rehydration liquid to match the capacity of the IPG gel strip. If too little rehydration liquid is added the IPG will under-rehydrate and the separation will be compromised. Similarly, if too much rehydration liquid is added and some of that liquid is not taken up by the IPG gel strip then proteins are lost in the liquid which is not taken up into the gel. Typically, high molecular weight proteins are preferentially lost in this process.

To overcome this drawback with passive rehydration some groups have advocated the use of rehydration trays with electrodes embedded in the troughs. The electrodes are used to provide a voltage (~50V) during the rehydration process. This electric field causes 'active' uptake of the proteins into the IPG gel matrix and results in more proteins entering the gel, especially high molecular weight proteins. However, if the rehydration solution comes in contact with both electrodes during the rehydration process, then the dissolved proteins may undergo electrophoretic transport to the electrodes in the free solution. If this occurs, a significant proportion of the proteins of the sample do not separate in the IPG because the sample proteins are transported to the electrode and then precipitate there. The

proteins which are lost in this process represent all molecular weights, not only high molecular weight proteins.

The present invention aims to provide an IPG gel strip rehydration tray that allows active rehydration to be done without the free rehydration solution coming into contact with the electrodes, thus preventing the electrophoretic transport of the proteins to the electrodes.

**Disclosure of Invention**

In a first aspect of the present invention there is provided an apparatus for rehydrating and for performing electrophoresis on a gel strip including:

(a) a tray defining at least one trough configured to receive a gel strip, said trough defining a centrally located rehydration area and an electrode area disposed either side of the rehydration area;

(b) means for delimiting the rehydration area of the trough from the electrode area; and

(c) electrode means including contact points adapted to contact either the gel strip in the electrode areas near the first and second end of the gel or a conducting or current carrying electrode bridge material which is in contact with the gel strip in the electrode areas, the electrode means being adapted to be connected to a means for supplying an electric current for imposing an electric potential in the strip between the electrodes.

Existing apparatus for rehydrating IPG gel strips all have troughs with flat bases or floors. Indeed the provision of a flat floor in troughs for rehydrating IPG gel strips is taught as being necessary for satisfactory rehydration. In contrast the inventors of the present invention have realised that having a floor or base in which the dehydration area is delimited from the electrode area by for example having a stepped floor or a wall or both. In such a manner active rehydration can be carried out without the rehydration solution coming into contact with the electrodes, thus preventing the electrophoretic transport of the proteins to the electrodes in free solution. In a preferred embodiment a means of preventing the rehydration solution from contacting the electrodes include small walls extending across the width of the trough and a relatively small air gap between the electrodes and the walls.

In a further preferred embodiment the gel in the rehydration area of the trough contacts a conducting/current carrying, electrode bridge which completes the circuit to contact point of the electrodes.

The electrode bridges may comprise filter paper or the like wetted with an electrically conducting liquid.

It is preferred that the electrode area is deeper than the rehydration area.

Typically the tray will define a plurality of substantially parallel troughs.

The trays may be designed to allow electrodes to contact the gel/electrode bridge assembly from above, thus eliminating the need for embedded electrodes in the troughs.

This arrangement lends itself to a disposable IPG gel strip rehydration and running tray. The ability to use a disposable, combined IPG gel strip rehydration and running tray overcomes a number of drawbacks with other commercially available systems.

The trays may be supplied with the dry IPG gel strips and dry electrode bridge material already in place in the grooves, thus eliminating the major handling step of setting up the trays. In addition, with disposable trays there is no problem with carryover from one sample to the next, whereas the current commercial trays require careful washing between uses.

The electrode assembly may include moulded pressure points, which rest on the gel strip where it overlaps the electrode bridge to ensure a good electrical contact between the gel strip and the electrode bridge.

The invention also encompasses a method of rehydrating and performing electrophoresis on a gel strip using the apparatus according to the present invention and/or its preferred embodiments.

In a related aspect the invention provides a method of rehydrating and performing electrophoresis on a gel strip comprising the steps of:

(a) providing a tray defining at least one trough with a gel strip, located in said trough, the trough defining a centrally located rehydration area and an electrode area disposed either side of the electrode area in which an absorbent electrode bridge is provided, the trough including means for delimiting the rehydration area of the trough from the electrode area;

(b) wetting the bridges with an electrically conducting liquid;

(c) adding rehydration liquid, containing a sample to be separated by electrophoresis to the trough;
(d) inserting a dry gel strip into the trough if a gel strip is not already present in the trough, the gel strip being longer than the rehydration area so that its ends rest on the electrode bridges;
(e) applying relatively low voltage across the gel strip the during a first period in which rehydration of the gel strip occurs;
(f) subsequently applying a relatively higher voltage to perform electrophoresis on the sample.

Typically the sample will be a mixture of macromolecules such as proteins, although other samples containing DNA, RNA, amino acids or other components which can be separated by electrophoresis may be used.

**Brief description of the Drawings**

The invention will now be described, by way of example only, and with reference to the accompanying drawings in which:-

Figure 1 is a schematic view of an apparatus for active rehydration of IPG gels;

Figure 2a shows an enlarged view of an electrode bridge area of the apparatus of Figure 1;

Figure 2b is a schematic side view of walls which delimit a rehydration area of the apparatus;

Figure 3 illustrates an electrode assembly inserted into the electrode bridge area of the apparatus;

Figures 4 and 4a are schematic sectional views along the length of grooves of further embodiments of the invention; and

Figure 5 is an isometric view of a yet further embodiment of the invention.

Figure 6 shows a gel image where the rehydration liquid containing a sample has been loaded passively, ie while not subject to an electric current; and

Figure 7 shows a gel image where the rehydration liquid containing a sample has been loaded actively, while subject to an electric current.

**Detailed Description of the Presently Preferred Embodiments**

Turning now to the drawings, Figure 1 shows a first embodiment of an apparatus 10 for rehydrating dry IPG gels with the aid of an applied electric field. In the embodiment shown in Fig. 1 the tray has ten elongate parallel grooves or troughs 12. However the tray could have more or less than ten grooves. The grooves shown in Figure 1 are 6mm wide, however in other embodiments the grooves they may be relatively narrower or relatively wider than 6mm.

Figure 2a shows an expanded view of one end of the tray in Figure 1 also shown schematically in Figure 2b. Each groove 12 has a base or floor 14 which is stepped at each end. Figure 2a shows only one end of each groove however both ends of each of the grooves are substantially identical. Each groove 12 defines a central rehydration area 16 at each longitudinal end of which there is a wall 18 which serves to contain the rehydration fluid within the designated rehydration area 18. The wall is about 1mm above the floor of the rehydration area. There is an electrode bridge region 20 on the opposite side of each wall 18. The electrode bridge region is 1mm below the floor of the rehydration area. The electrode bridge area may typically be 20mm long and in use, as is discussed below, is sufficiently long to define an air gap 22 (Fig. 2b) between a piece of filter paper 23 or the like forming part of the bridge and the wall 18 which retains the rehydration solution. This prevents capillary movement from the rehydration area onto the electrode bridge region 20. The dimensions given above are however exemplary only and might be varied.

The grooves which are typically 6mm wide allow room for loading the rehydration solution when an IPG gel strip is already in place in the groove, such as in the disposable tray format discussed above. Standard commercially available IPG gel strips are 3.3mm wide, thus the 6mm wide grooves will also allow for the use of relatively wider non-standard IPG gel strips up to approximately 5mm wide.

The length of the grooves/trays may vary depending on the length of commercially available IPG gel strips which are to be used in the tray. Commercially available IPG gel strips are usually 7, 11, 13, 17 or 18cm long.

The rehydration area may be approximately 5mm shorter than the respective IPG gel strip to be rehydrated, to allow overlap of the IPG gel strip 29 (refer to Figure 2b) into the electrode bridge area. The length of the electrode bridge area is 20mm. In a preferred embodiment the electrode

bridge area contains a 6mm X 20mm piece of 2mm thick filter paper 23 which fills the electrode bridge area except for a small air gap 22. A piece of filter paper that size requires between 50 and 200μL of water to become slightly hydrated. The quantity of water in the electrode bridge area requires precise control, to allow electrical contact without excessive wetness, which would cause a disturbance in the separation.

In other embodiments of the design the electrode bridge area and/or the rehydration area could be scaled up or down in size to accommodate different requirements.

Figure 3 shows an expanded view of one end of the tray in Figure 1 illustrating an external electrode assembly 24 being lowered into the electrode bridge areas of the grooves.

The external electrode assembly 24 consists of a series of pressure blocks 26 (which are dark grey in Figure 3) which rest/press on the IPG gel strip to ensure good contact between the IPG gel strip and the electrode bridge material and an electrode itself 28 (which is shown in light grey in Figure 3). The electrode element comprises a series of electrode elements 30 located at the end of each pressure block which are integral with a joining element 32 linking the electrode elements together. The electrode elements are located near the outer edge of the trough to make full use of the length of the electrode bridge material.

The apparatus of the present invention allows for active loading of a rehydration liquid containing a biological sample to be separated into the gel strip for electrophoresis, ie loading while the current is running. Further it allows the use of filter paper or the like as an electrode bridge while the active loading is happening without getting the rehydration liquid onto the paper.

Figure 4a illustrates an alternative embodiment of the invention in which the base of the groove is curved along its length and in its centre, measured along the longitudinal direction or length of the groove defines a laterally extending loading channel 42 having a depth of 0.5mm and a width w, measured in the longitudinal direction of the groove, of 2mm. The curved base allows the maximum depth of the groove at its longitudinal centre to be 2mm, so it can contain more rehydration liquid. The curved floor of the rehydration area supports the IPG strip well. The height h of the end walls is 0.5mm.

Figure 4b shows a variant of the design of Figure 4a in which the floor of the rehydration are is flat and there are two sloping end sections 44 at the ends of the rehydration are which slope at about 30 degrees. The channel 42 is identical to that of Figure 4b.

Figure 5 illustrates a yet further variant of the invention in which two parallel walls 18 delimit the ends of the rehydration area 16 of the grooves. This helps to maintain an air gap between the rehydration solution and the electrode bridge area.

Comparative Example

Figure 6 shows a gel image where the rehydration liquid containing a sample of Human red blood cells has been loaded passively, ie while not subject to an electric current. The sample was prepared according to the method of Beutler, E., West, C., Blume, K-G.. *J. Lab. Clin. Med.* 1976, **88**, 328-333. The membrane samples were then solubilised in a solution containing 7M urea, 2M thiourea, 1% C7Bz0 (a detergent) and 3mM tributyl phosphine. The solubilised protein was then loaded (passively) onto the dry IPG strip by rehydration in the case of the example shown in Figure 6 without active rehydration.

Example

Figure 7 shows a gel image where the rehydration liquid containing a sample of Human red blood cells prepared according to the example set out above has been loaded actively, while subject to low voltage of about 50 volts. As can be seen in comparison with the gel shown in Figure 6 the final gel strip shown in Figure 7 is better than the passively loaded strip because there are more distinct protein spots resolved. Also the large amount of smearing which is apparent in figure 6 is not present on Figure 7. Further high molecular weight proteins are better resolved on the active gel. It is possible to identify "spectrin" (50) a very large 250kDa prtein on the gel of Figure 7. Spectrin is a signature protein of RBC membranes and is very difficult to resolve.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:

1. An apparatus for rehydrating and for performing electrophoresis on a gel strip including:-

(a) a tray defining at least one trough configured to receive a gel strip, said trough defining a centrally located rehydration area and an electrode area disposed either side of the rehydration area;

(b) means for delimiting the rehydration area of the trough from the electrode area; and

(c) electrode means including contact points adapted to contact either the gel strip in the electrode areas near the first and second end of the gel strip or a conducting or current carrying electrode bridge material which is in contact with the gel strip in the electrode areas, the electrode means being adapted to be connected to a means for supplying an electric current for imposing an electric potential in the strip between the electrodes.

2. An apparatus as claimed in any preceding claim wherein the means for delimiting the rehydration area of the trough from the electrode area include walls extending laterally across the width of the trough and an air gap defined between each electrode means and the wall adjacent said electrode means.

3. An apparatus as claimed in any preceding claim wherein two spaced apart parallel walls extend across the trough defining a gap there between

4. An apparatus as claimed in claim 2 or 3 wherein a part of the gel strip in the rehydration area of the trough adjacent the delimiting wall contacts a conducting/current carrying, electrode bridge.

5. An apparatus as claimed in claim 4 wherein the electrode bridges comprise an absorbent material wetted with an electrically conducting liquid.

6. An apparatus as claimed in claim 5 wherein the absorbent material is paper.

7. An apparatus as claimed in any preceding claim wherein the electrode area is deeper than the rehydration area.

8. An apparatus as claimed in any preceding claim wherein a laterally extending channel is defined in a floor of the groove.

9. An apparatus as claimed in any preceding claim wherein the trough does not include embedded electrodes and the electrodes contact the electrode bridge material from above.

10. An apparatus as claimed in any one of claims 1 to 8 wherein the tray includes a dry IPG gel strip and dry electrode bridge material located in place in the trough.

11. An apparatus as claimed in any preceding claim further including pressure applying means which rest on the gel strip where the strip overlaps the electrode bridge material to ensure a good electrical contact between the gel strip and the electrode bridge material.

12. An apparatus as claimed in any preceding claim wherein the tray defines a plurality of substantially parallel troughs.

13. A method of rehydrating and performing electrophoresis on a gel strip using the apparatus according to the any of the preceding claims.

14. A method of rehydrating and performing electrophoresis on a gel strip comprising the steps of:

(a) providing a tray defining at least one trough with a gel strip, located in said trough, the trough defining a centrally located rehydration area and an electrode area disposed either side of the electrode area in which an absorbent electrode bridge is provided, the trough including means for delimiting the rehydration area of the trough from the electrode area;

(b) wetting the bridges with an electrically conducting liquid;

(c) adding rehydration liquid, containing a sample to be separated by electrophoresis into the centrally located rehydration area of the trough;

(d) inserting a dry gel strip into the trough if a gel strip is not already present in the trough, the gel strip being longer than the rehydration area so that its ends rest on the electrode bridges;

(e) applying relatively low voltage across the gel strip the during a first period in which rehydration of the gel strip occurs;

(f) subsequently applying a relatively higher voltage to perform electrophoresis on the sample.

15. The method of claim 14 wherein the sample is a mixture of macromolecules selected from the group consisting of protein samples containing DNA, RNA, amino acids or other components which can be separated by electrophoresis may be used.

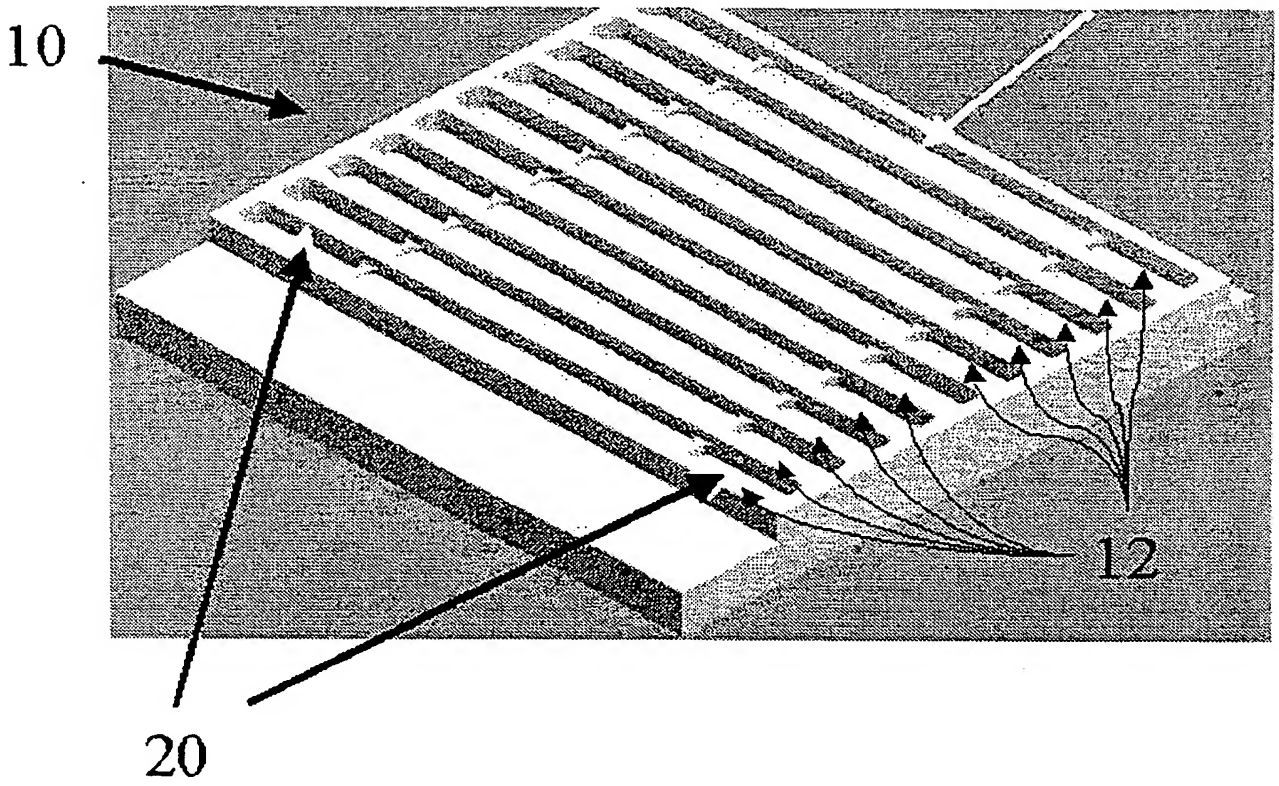



Figure 1

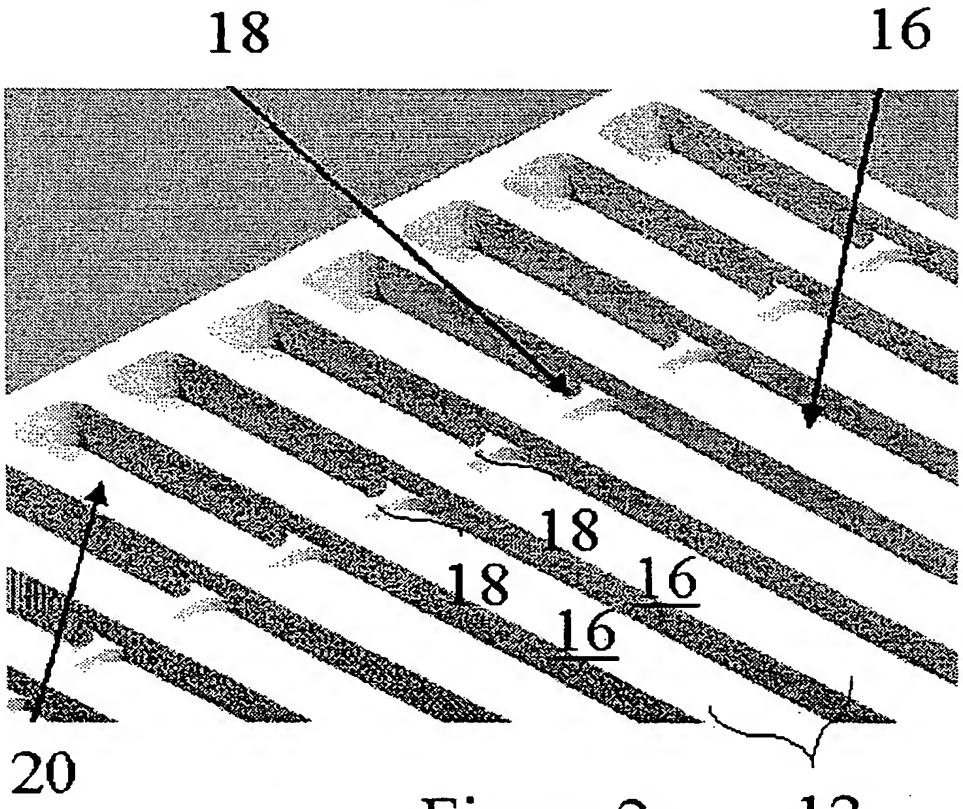



Figure 2a

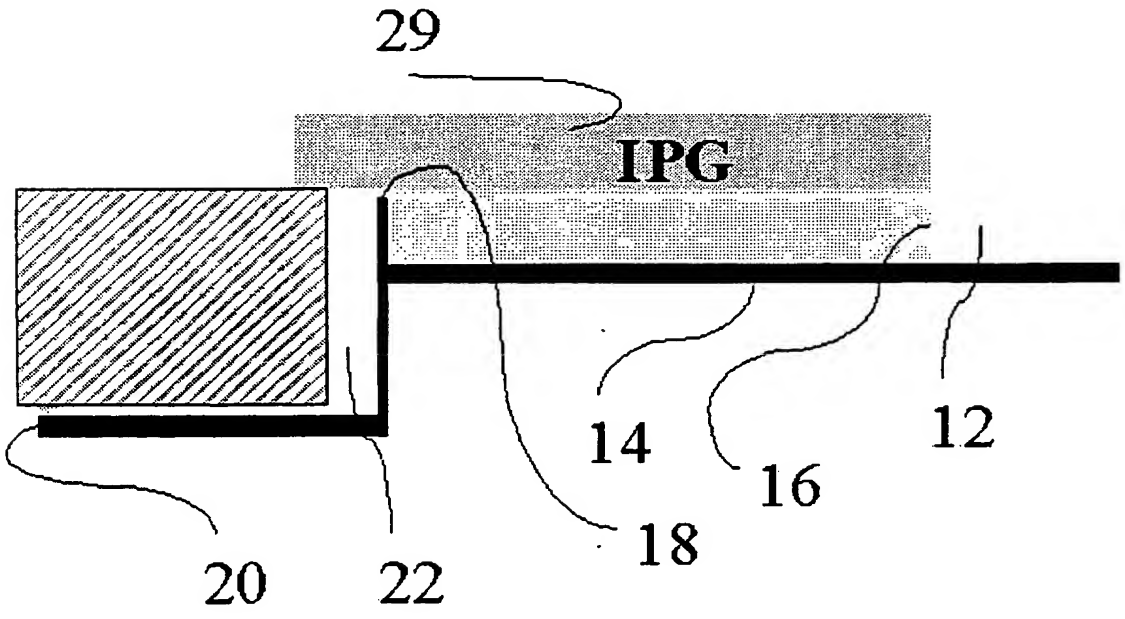



Figure 2b

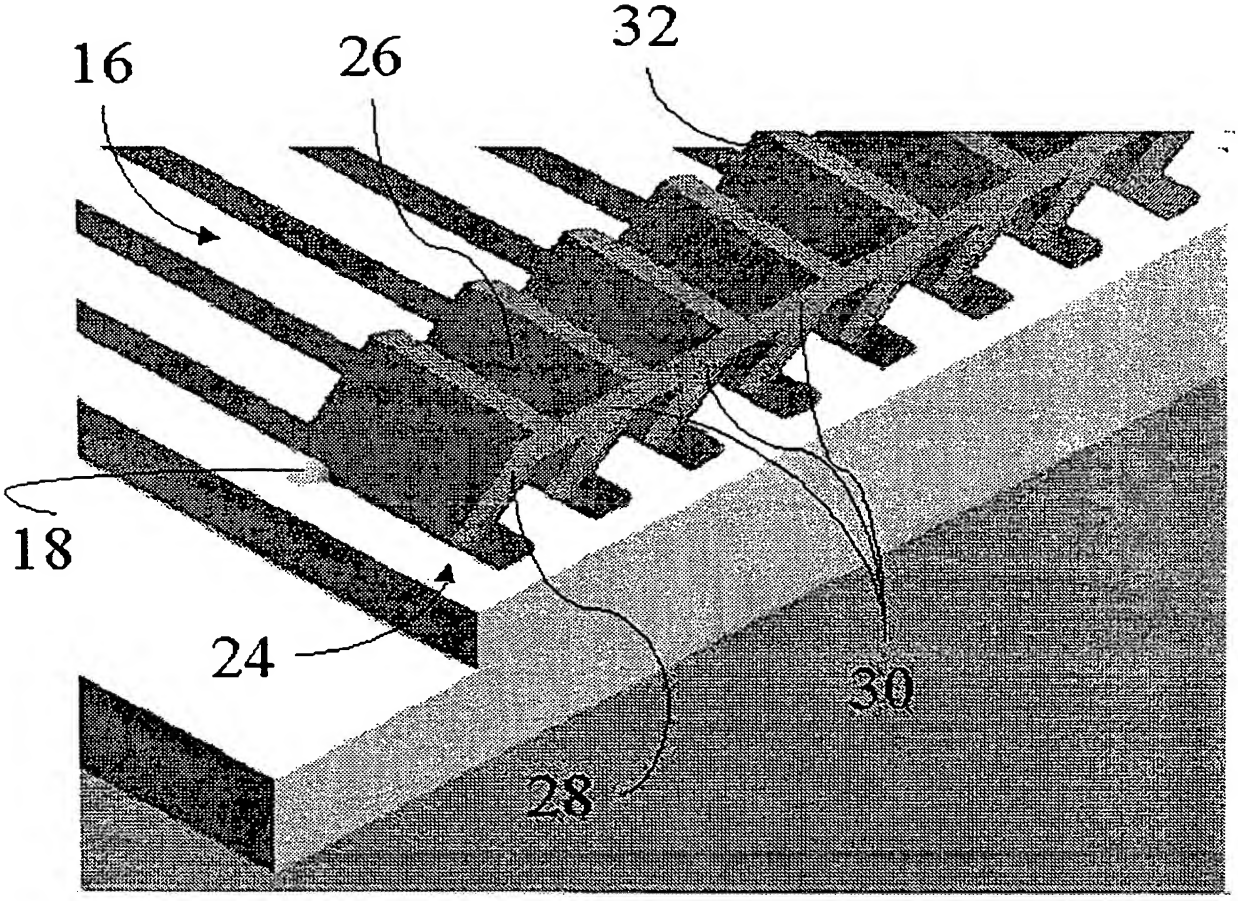



Figure 3

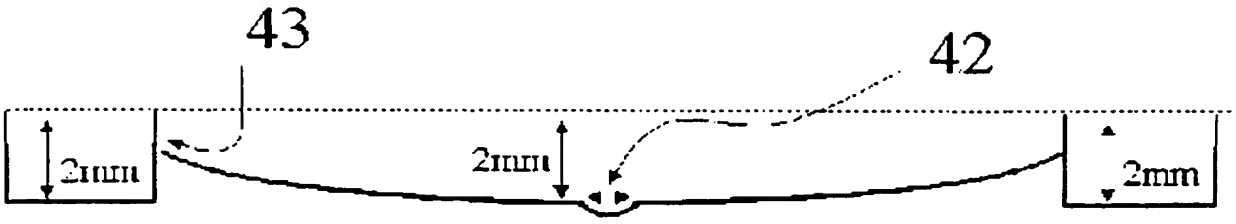



Figure 4a 40

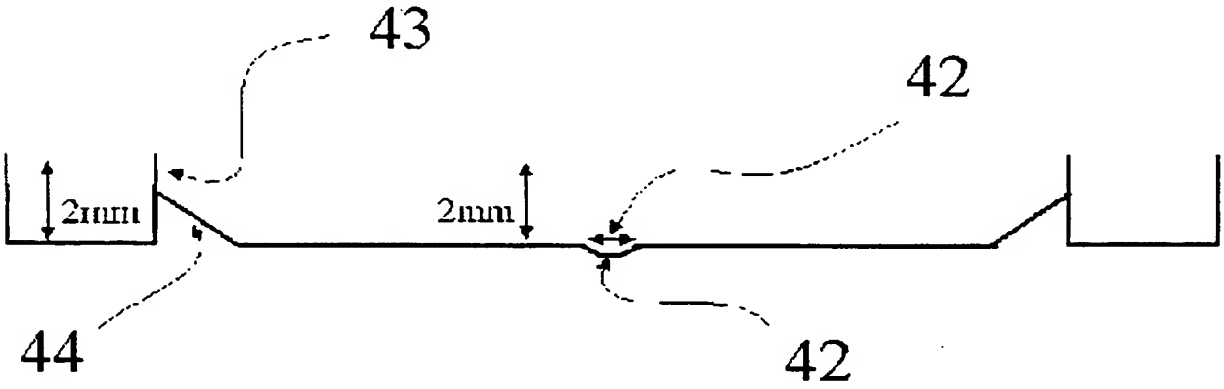



Figure 4b

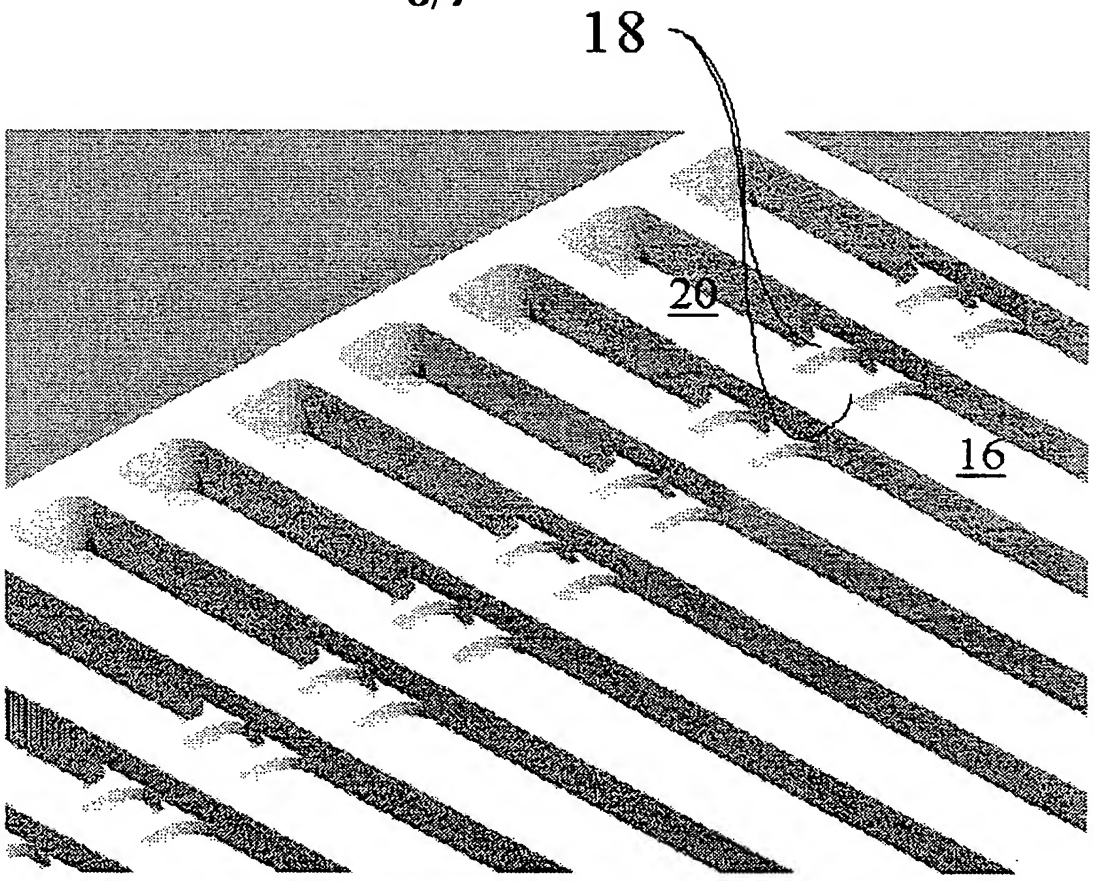



Figure 5

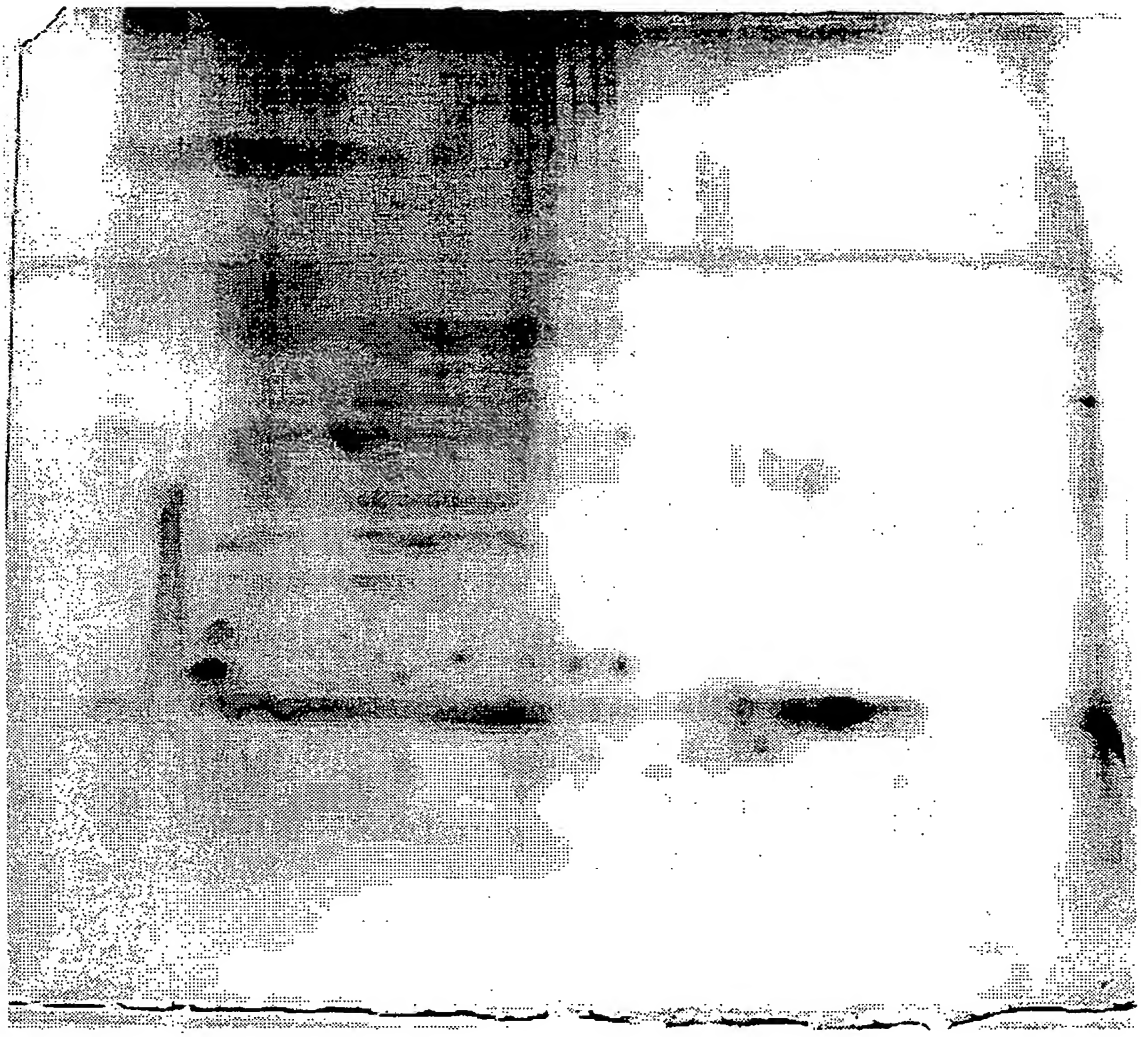


Figure 6

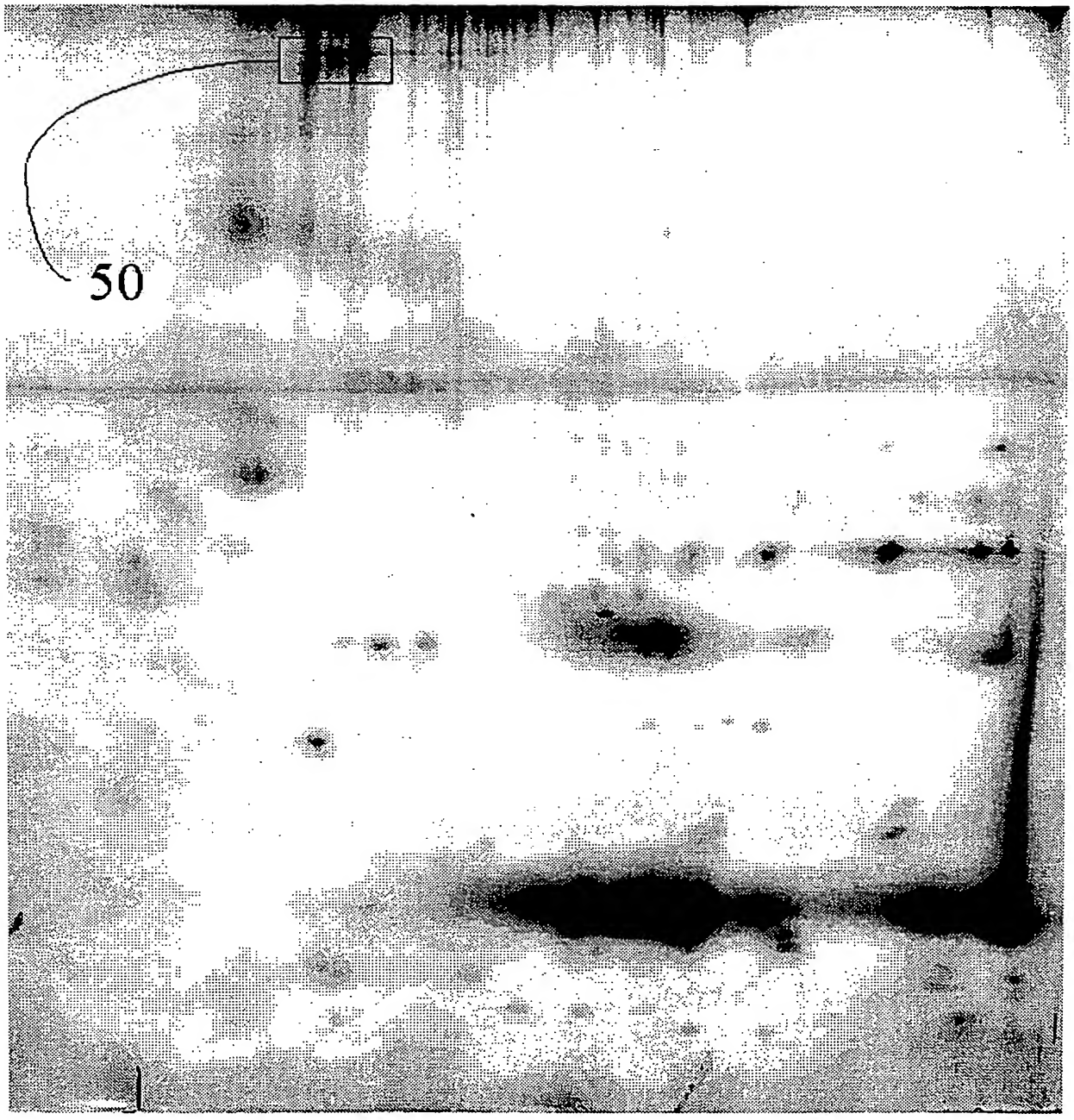



Figure 7